510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

K041238

B. Purpose for Submission:

Premarket notification

C. Measurand:

Human IgG antibodies to *Helicobacter pylori*

D. Type of Test:

Enzyme linked immunosorbent (ELISA) assay for qualitive detection of IgG antibodies to *Helicobacter pylori*

E. Applicant:

Biohit Plc

F. Proprietary and Established Names:

Helicobacter pylori IgG ELISA Test

G. Regulatory Information:

1. Regulation section:

   866.3110

2. Classification:

   I

3. Product code:

   LYR - Campylobacter pylori

4. Panel:

83 Microbiology

H. Intended Use:

1. Intended use(s):

   The *Helicobacter pylori* IgG ELISA Test is an enzyme linked immunosorbent (ELISA) assay for qualitive detection of human IgG class antibodies to *Helicobacter pylori* (*H. pylori*) in EDTA or heparin treated plasma and in serum.

2. Indication(s) for use:

   The test is indicated for use as an aid in the diagnosis of *H. pylori* infection in adult patients with clinical symptoms of gastritis.

3. Special conditions for use statement(s):

   Prescription use

4. Special instrument requirements:

   Microplate reader, 450 nm

I. Device Description:

The *H. pylori* IgG antibodies ELISA test is an enzyme immunoassay technique utilizing a partially purified *H. pylori* antigen adsorbed on a polystyrene coated strip on a microplate and a detection antibody (Conjugate Solution) labeled with horseradish peroxidase (HRP) to detect antibodies to *H. pylori* in plasma or serum. The Substrate Solution contains an tetramethylbenzidine (TMB) which is oxygenated by the enzyme in the Conjugate to produce a blue end-product. The Stop Solution (with sulfuric acid) stops the enzyme reaction, and turns the substrate yellow if antibody is present in the patient serum. The kit also includes a Washing Buffer (phosphate), Diluent Buffer (phosphate) and four Incubation Covers (plastic sheets) to cover the microplate during incubation.

A Calibrator, containing 1.5 mL of human serum-based *H. pylori* IgG, is used as a functioning calibrator, to determine if the assay is functioning appropriately. Negative and Positive Controls made of human serum-based *H. pylori* IgG are included. The kit contains 12 microplates with 8 coated strips in a frame. The reagents are sufficient for 96 wells.

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J. Substantial Equivalence Information:

1. Predicate device name(s):

   IMMULITE® *Helicobacter pylori IgG*

2. Predicate 510(k) number(s):

   k000463

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended use:	For detection of *H. pylori* IgG antibodies	Same
Assay	Qualitative	Same
Sample	Serum and/or Plasma	Serum

Differences		
Item	Device	Predicate
Technology	ELISA	Chemiluminescent EIA
Incubation	90 minutes	65 minutes
Reader	Microplate Reader 450 nm	Luminometer

K. Standard/Guidance Document Referenced (if applicable):

FDA document: Review Criteria for Assessment of Laboratory Tests for the Detection of Antibodies to *Helicobacter pylori*

L. Test Principle:

The *H. pylori* IgG antibodies ELISA test is based on an enzyme immunoassay technique utilizing a partially purified *H. pylori* antigen adsorbed on a microplate and a detection antibody labeled with horseradish peroxidase (HRP). If *H. pylori* antibodies are present in the sample they bind with the purified *H. pylori* antigen coated on the polystyrene wells of the microplate. Wells are then washed to remove residual particles. An HRP-conjugated monoclonal anti-human IgG is added to bind the *H. pylori* antibodies, the wells are washed and a TMB substrate is added. The substrate is oxygenated by the enzyme and a blue colored end product is produced. The enzyme reaction is terminated with the stop solution. The plates are read on a microplate reader. *H. pylori* positive samples turn yellow with EIU units of > 42.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Reproducibility was established at three sites using a panel consisting of a high positive, 3 boderline positives, and 2 negative samples. Inter-assay and intra-assay variation was determined. For serum samples, % CV for inter-assay ranged from 1.5 -6.7%. EDTA- plasma% CV for inter-assay ranged from 2.5 – 7.0%, and heparin from 1.3 – 6.6%. For intra-assay, % CV ranged from 2.5 – 9.8 for serum, 1.3 – 11.8 % for EDTA-plasma, and 1.3 – 19.2% for heparin. These values are similar to other ELISA *H. pylori* assays.

   b. Linearity/assay reportable range:

   Not Applicable

   c. Traceability, Stability, Expected values (controls, calibrators, or methods):

   Stability studies were conducted to stress the assay and samples.

   Positive and Negative Controls are included in the kit and were evaluated during the studies. The Calibrator included is only for verifying that the assay is functioning properly. A value $\geq$ 1.000 is assigned

   d. Detection limit:

   The detection limit was determined by evaluating serial dilutions performed on serum, EDTA-plasma, and heparin-plasma samples. Values obtained gave a 10% CV limit and EIU values for serum of 4.0, for < 1.3 for EDTA-plasma, and 4.2 for heparin-plasma samples. Additionally, 24 zero blank replicates were assayed and gave values within a 2 standard deviation range. The mean value was 2.3 EIU.

   e. Analytical specificity:
   Cross-reactivity: To determine if the presence of closely related microorganisms would affect the specificity of the assay, a panel consisting of 4 strains of Yersinia, E. coli, Campylobacter jejuni, and 2 strains of Salmonella, all at concentrations >108 CFU/ml. There were no false positive or false negative results observed.

   Interference Study: Assay was evaluated for triglycerides using ten samples spiked with triglyceride, and hemoglobin and bilirubin interference using 20

spiked samples. Reactivity or non-reactivity was not affected by increasing concentrations of none of these substances.

f. Assay cut-off:

The cut-off was determined in a case control study using 29 patient samples that had histology results available for normal stomach mucosa without evidence of *H. pylori* infection, or non-atrophic or superficial gastritis without *H. pylori* infection. The cut-off was further challenged in a study using histology and culture as the "gold" standard and another ELISA assay for comparison.

2. Comparison studies:

a. Method comparison with predicate device:

Biohit H. pylori	**Histology**	
	Pos	**Neg**
Pos	144	79
Neg	34	247
Percent Positive Agreement = 80.9% Percent Negative Agreement = 75.8%		

b. Matrix comparison:

Anticoagulant studies were conducted to determine if there were significant differences between when using serum, EDTA-plasma, and heparin-plasma specimens. No significant differences were observed between serum and EDTA; however heparin-plasma samples showed wider variation in results.

3. Clinical studies:

a. Clinical Sensitivity:

The Biohit *H. pylori* IgG antibodies ELISA test was evaluated for clinical sensitivity using a total of 264 samples. The samples were compared to of biopsied gastric mucosa culture. The results were as follows:

		Culture	
		+	-
Biohit H.P.	Pos.	108	51
	Neg	10	95

Clinical Sensitivity = 91.5%

(95% CI = 86.3 – 95.0%)

*b. Clinical specificity**:***

Clinical Specificity = 65.1%

(95 % CI – 60.8 – 68.0%)

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off**:**

The assay cutoff was validated during the clinical studies. Only patients with histology and culture procedures available were used in validating the reactive cutoff. The optimal cutoff was obtained using a Receiver-Operating Curve (ROC) analysis for maximizing the sum of the sensitivity and specificity of the assay. The cutoff was determined to be 38.5 EIU.

5. Expected values/Reference range:

In the studies conducted to demonstrate that this device is substantially equivalent to the predicate device, when compared to culture, the assay had a Predictive Positive Value of 67.9% and a Predictive Negative Value of 90.5%. However, antibody levels do not necessarily correlate to infection. *H. pylori* positive samples are expected to give values of > 42 EIU.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.